

PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Applicant:	Watkins, Jeffry D.	Group Art Unit:	1644
Serial No.:	10/553,938	Examiner:	Ron Schwadron, Ph.D.
Application Date:	October 21, 2005	Confirmation No.:	8652
For:	CD20 Binding Molecules		
Docket No.:	X-16760A		

DECLARATION OF APPLICANT UNDER 37 C.F.R. § 1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Jeffry D. Watkins, hereby declare the following:

A duly executed Declaration and Power of Attorney was filed on October 21, 2005 in the above-referenced patent application, declaring that Julian Davies, David M. Marquis, Barrett W. Allan, Brian Ondek, and myself are the original and first inventors of the subject matter which is claimed and for which a patent is sought in the above-referenced patent application, and the same is true and correct.

I understand that a Business Wire article entitled "Applied Molecular Evolution Advances Optimized Versions of anti-TNF alpha and anti-CD20 Monoclonal Antibody Therapeutic Candidates", dated January 3, 2003 (hereinafter, the "Business Wire reference") has been cited by the Examiner in the above-referenced patent application as anticipating the pending claims of the same.

I have also read and understand the Business Wire reference. In relevant part, the Business Wire reference describes, in entirely functional terms, a CD20 binding antibody,

AME-133, reported to have improved functional attributes as compared to Rituxan®, a therapeutic CD20 binding antibody known and commercialized at the time of the publication of the Business Wire reference.

I also understand that the above-referenced patent application presently claims compositions comprising a CD20 binding molecule (e.g., antibodies or CD20 binding fragment thereof) comprising a set of three structurally defined heavy chain CDRs and a set of three structurally defined light chain CDRs.

The invention presently claimed by the above-referenced patent application was based on detailed experiments involving antibody optimization which resulted in functionally improved CD20 binding antibodies comprising the CDRs defined by specific amino acid sequences.

This declaration is to establish the actual reduction to practice in the United States of the invention claimed in the above-referenced patent application, at a day prior to January 3, 2003, which is the effective date of the Business Wire reference.

At the relevant time of the actual reduction to practice of the invention claimed by the above-referenced application, I held the position of Chief Scientific Officer at Applied Molecular Evolution (AME), Inc.

The invention disclosed and claimed in the instant patent application was reduced to practice (in the United States) prior to January 3, 2003, as is evidenced by the attached Exhibits, which are more fully described below.

To the best of my knowledge and belief, the claimed invention was not sold or in public use in the United States for one year prior to the date of the above application.

To the best of my knowledge and belief, the claimed invention was not patented nor described in a printed publication in such a manner that a person of ordinary skill in the field of the invention would have been able to make or use the claimed invention, without undue experimentation, prior to the date of the above application.

The attached Exhibits were generated and/or prepared by AME employees, including Christine Hawelka and Ying Nie, who are not inventors of the claimed subject matter, while recording and/or documenting work conducted (in the United States) under the direction and supervision of myself and/or the other inventors listed on the Declaration and Power of Attorney filed on October 21, 2005.

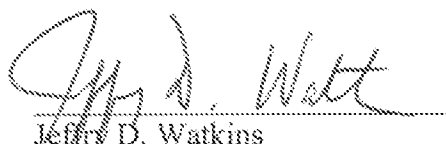
The following documents are submitted as evidence establishing the date of completion of the claimed invention of the above-referenced application as being prior to January 3, 2003.

- a. Copies of sequence files generated with SequencherTM (Gene Codes Corp.) on October 10, 2002 showing sequences encoding heavy chain variable regions and light chain variable regions of CD20 binding molecules, including those of AME 33 (Exhibits 1-4).
- b. Copies of various pages from Christine Hawelka's AME Research Notebook #585, dated from October 9, 2002 to October 17, 2002 (Exhibit 5).
- c. Copies of the cover page and page 19 (dated November 4, 2002) from Ying Nie's AME Research Notebook #613 (Exhibit 6).

The documents submitted as Exhibits 1-6 clearly demonstrate that the presently claimed invention was reduced to practice (in the United States) on or before October 10, 2002.

The documents submitted as Exhibits 1-6 clearly demonstrates that the presently claimed invention was reduced to practice (in the United States) prior to January 3, 2003, the effective date of the Business Wire reference.

I further declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willfully false statements may jeopardize the validity of this application or any patent issuing thereon.


Jeff D. Watkins

June 29, 2011
Date

DESCRIPTION OF THE EXHIBITS

Exhibit 1

A copy of an electronic sequence file generated with SequencherTM software (Gene Codes Corp.) on October 10, 2002 showing the sequences (both nucleotide and amino acid sequences) obtained from DNA sequencing of the DNA encoding the heavy chain variable regions of various CD20 binding molecules. In particular, the nucleotide sequence encoding the heavy chain variable region of the CD20 binding molecule AME 33 is shown as the fourth nucleotide sequence down from the top of the page (i.e., 1_DM.33.530_F04_12.abl). Directly below the fourth DNA sequence is the amino acid sequence (provided in conventional single-letter code) of the heavy chain variable region of the CD20 binding molecule AME 33.

Exhibit 2

A copy of an electronic sequence file generated with SequencherTM software (Gene Codes Corp.) on October 10, 2002 showing the sequences (both nucleotide and amino acid sequences) obtained from DNA sequencing of the DNA encoding the light chain variable regions of various CD20 binding molecules. In particular, the nucleotide sequence encoding the light chain variable region of the CD20 binding molecule AME 33 is shown as the fourth nucleotide sequence down from the top of the page (i.e., 1_DM.33.355_F05_11.abl). Directly below the fourth DNA sequence is the amino acid sequence (provided in conventional single-letter code) of the light chain variable region of the CD20 binding molecule AME 33.

Exhibit 3

A marked up version of Exhibit 1 has been provided here as Exhibit 3 for the Examiner's convenience.

The amino acid sequences of CDRH1, 2, and 3 of the CD20 binding molecule AME 33 are underlined at page 2, 3, 4 and 5, respectively, of Exhibit 3. The underlined amino acid sequences of CDRH1, 2, and 3 correspond exactly to SEQ ID NOs: 25, 39, and 57, respectively, of the above-referenced application. Importantly, SEQ ID NOs: 25, 39, and 57 are all recited elements in present claim 34.

Furthermore, in Exhibit 3, the amino acid sequence of the entire heavy chain variable region of the CD20 binding molecule AME 33 has been enclosed by brackets (starting at page 1 and ending at page 5). The entire heavy chain variable region of the CD20 binding molecule AME 33 (shown enclosed in brackets) corresponds exactly to SEQ ID NO: 61 of the above-referenced application. Importantly, SEQ ID NO:61 is a recited element in present claim 48.

Exhibit 4

A marked-up version of Exhibit 2 has been provided here as Exhibit 4 for the Examiner's convenience.

The amino acid sequences of CDRL1, 2, and 3 of the CD20 binding molecule AME 33 are underlined at page 2, 3, and 4, respectively, of Exhibit 4. The underlined amino acid sequences of CDRL1, 2, and 3 correspond exactly to SEQ ID NOs: 5, 13, and 19, respectively, of the above-referenced application. Importantly, SEQ ID NOs: 5, 13, and 19 are all recited elements in present claim 34.

Furthermore, in Exhibit 4, the amino acid sequence of the entire light chain variable region of the CD20 binding molecule AME 33 has been enclosed by brackets (starting at page 1 and ending at page 5). The entire light chain variable region of the CD20 binding molecule AME 33 (shown enclosed in brackets) corresponds exactly to SEQ ID NO:59 of the above-referenced application. Importantly, SEQ ID NO:59 is a recited element in present claim 48.

Exhibit 5

A copy of the cover page, table of contents page, and various other pages from Christine Hawelka's AME Research Notebook #585, dated from October 9, 2002 to October 17, 2002, are provided as Exhibit 5. Throughout these research notebook pages, references to "33", or variations thereof (such as "#33", "33 F1", etc.), are references to the CD20 binding molecule referred to as AME 33 in the above-referenced application.

Page 66 of Christine Hawelka's AME Research Notebook #585, indicates, *inter alia*, that David Marquis ("Dave") set up and finished high-titer ("HT") and ("single-strand") preps of various clones ("31-40"), including clone 33, on October 8, 2002 and October 9, 2002, respectively.

Page 68 of Christine Hawelka's AME Research Notebook #585, describes, *inter alia*, a fixed Ramos cell ELISA binding assay with various CD20 binding molecules, including Fab 33. It is noted on this notebook page, dated October 10, 2002, that "#32, 33, 35, [and] 40 look the best, so will investigate them further". For the avoidance of doubt, the references to "33" or "reference is referred to as AME 33, Fab AME 33 or the like in the present application.

Page 73 of Christine Hawelka's AME Research Notebook #585, indicates, *inter alia*, a fixed Ramos cell ELISA binding assay with various CD20 binding molecules, including the Fab AME 33. The page, dated October 17, 2002, notes that after 18 hours, "Brian's 4H5 as well as 33 [and] 40 staying on pretty well".

Exhibit 6

A copy of the cover page and page 19 Ying Nie's AME Research Notebook #613 are provided as Exhibit 6. Page 19 of Ying Nie's AME Research Notebook #613, dated November 4, 2002, documents the purification of single-strand DNA ("ssDNA") encoding the light chain variable region of clone 33 ("VL33") and the heavy chain variable region of clone 33 ("VH33"). "VL33" and "VH33" on page 19 of this notebook are refer to DNA molecules encoding the light chain variable region and heavy chain variable region, respectively, of the CD20 binding molecule referred to as AME 33 in the above-referenced application.

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*+ 's 1D10 comb1 10-10-02.SPF"
sequencer (cm)
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1_DM.31.530_H04_16.ab1	#1	GTGAACAACAAGCACTATTGGCACTGGCACTCTTACCGTTACTGTTACACCTGTGCGAATAAGCCGAGGTGCAGCTGCTGCAGTCTGGAGCA
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	V	K Q S T I A L A L L P L L F T P V A K A E V Q L V Q S G A
1_DM.33.530_F04_12.ab1	#1	GTGAACAACAAGCACTATTGGCACTGGGCACTCTTACCGTTACTGTTACACCTGTGCGAATAAGCCGAGGTGCAGCTGCTGCAGTCTGGAGCA
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	V	K Q S T I A L A L L P L L F T P V A K A E V Q L V Q S G A
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	V	K Q S T I A L A L L P L L F T P V A K A E V Q L V Q S G A
<hr/>		
	#1	GTGAACAACAAGCACTATTGGCACTGGCACTCTTACCGTTACTGTTACACCTGTGCGAATAAGCCGAGGTGCAGCTGCTGCAGTCTGGAGCA
	V	K Q S T I A L A L L P L L F T P V A K A E V Q L V Q S G A

ContigVH
Sequencher (tm) "+"s 1D10 combi 10-10-02.SPF"

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ContlgvH
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ContigVH
Sequencher(tm) '+'s ID10 combi 10-10-02.SPF"

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ContigvH
Sequencher(tm) '+'s ID10 comb1 10-10-02.SPf"

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	T	X V G G D W T F D V W G K G T T V T V S S A S T K G
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	T	X V G G D W Q F D V W G K G T T V T V S S A S T K G
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ContigVL
Sequencher (tm) *+s ID10 combi 10-10-02.SPF*

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Sequencher(tm)  *+s 1D10 combi 10-10-02.SPF"
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1010 VL	#88	CTGCTCTTGTCTCCAGGGGAAAGAGCCACCCTCTCTCTGACAGGGGCCAGCTCAAGTGTACCGGTACATCCCACTGGGTACAGCAGAAACCT	L S L S P G E R A T L S C R A S S S V F Y I H W Y Q Q K P
1.DM.31.355_H05_15.ab1	#88	CTGTCTTTTGTCTCCAGGGGAAAGAGCCACCCTCTCTCTGACAGGGGCCAGCTCAAGTGTACCGGTACATCCCACTGGGTACAGCAGAAACCT	L S L S P G E R A T L S C R A S S S V F Y I H W Y Q Q K P
1.DM.32.355_G05_13.ab1	#88	CTGTCTTTTGTCTCCAGGGGAAAGAGCCACCCTCTCTCTGACAGGGGCCAGCTCAAGTGTACCGGTACATCCCACTGGGTACAGCAGAAACCT	L S L S P G E R A T L S C R A S S S V F Y I H W Y Q Q K P
1.DM.33.355_F05_11.ab1	#88	CTGTCTTTTGTCTCCAGGGGAAAGAGCCACCCTCTCTCTGACAGGGGCCAGCTCAAGTGTACCGGTACATCCCACTGGGTACAGCAGAAACCT	L S L S P G E R A T L S C R A S S S V F Y I H W Y Q Q K P
1.DM.34.355_E05_09.ab1	#88	CTGTCTTTTGTCTCCAGGGGAAAGAGCCACCCTCTCTCTGACAGGGGCCAGCTCAAGTGTACCGGTACATCCCACTGGGTACAGCAGAAACCT	L S L S P G E R A T L S C R A S S S V F Y I H W Y Q Q K P
1.DM.35.355_D05_07.ab1	#88	CTGTCTTTTGTCTCCAGGGGAAAGAGCCACCCTCTCTCTGACAGGGGCCAGCTCAAGTGTACCGGTACATCCCACTGGGTACAGCAGAAACCT	L S L S P G E R A T L S C R A S S S V F Y I H W Y Q Q K P
1.DM.36.355_C05_05.ab1	#88	CTGTCTTTTGTCTCCAGGGGAAAGAGCCACCCTCTCTCTGACAGGGGCCAGCTCAAGTGTACCGGTACATCCCACTGGGTACAGCAGAAACCT	L S L S P G E R A T L S C R A S S S V F Y I H W Y Q Q K P
1.DM.39.355_B05_03.ab1	#88	CTGTCTTTTGTCTCCAGGGGAAAGAGCCACCCTCTCTCTGACAGGGGCCAGCTCAAGTGTACCGGTACATCCCACTGGGTACAGCAGAAACCT	L S L S P G E R A T L S C R A S S S V F Y I H W Y Q Q K P
1.DM.40.355_A05_01.ab1	#88	CTGTCTTTTGTCTCCAGGGGAAAGAGCCACCCTCTCTCTGACAGGGGCCAGCTCAAGTGTACCGGTACATCCCACTGGGTACAGCAGAAACCT	L S L S P G E R A T L S C R A S S S V F Y I H W Y Q Q K P
	#88	CTGTCTTTTGTCTCCAGGGGAAAGAGCCACCCTCTCTCTGACAGGGGCCAGCTCAAGTGTACCGGTACATCCCACTGGGTACAGCAGAAACCT	L S L S P G E R A T L S C R A S S S V F Y I H W Y Q Q K P

ContigVL
Sequencher (cm) "+"s 1D10 combi 10-10-02.SPF"

#175 1D10 VL
G G C C A G G C T C C C A G G C T C T C A T C T A T G C C A C A T C C A C C T G G C T T C T G G C A T C C C A G A C A G G T T C A G T G G C A G T G G G T C T G G G A C A
G Q A P R L L I Y A T S N L A S G I P D R F S G S G T
#175 1_DM.31.355_H05_15.ab1
G G C C A G G C T C C C A G G C T C T C A T C T A T G C C C G T T C C A A C C T G G C T T C T G G C A T C C C A G A C A G G T T C A G T G G C A G T G G G T C T G G G A C A
G Q A P R L L I Y A R S N L A S G I P D R F S G S G T
#175 1_DM.32.355_G05_13.ab1
G G C C A G G C T C C C A G G C T C T C A T C T A T G C C A C A T C C G C T C T G G C T T C T G G C A T C C C A G A C A G G T T C A G T G G C A G T G G G T C T G G G A C A
G Q A P R L L I Y A T S A L A S G I P D R F S G S G T
#175 1_DM.33.355_F05_11.ab1
G G C C A G G C T C C C A G G C T C T C A T C T A T G C C A C A T C C G C T T G G C T T C T G G C A T C C C A G A C A G G T T C A G T G G C A G T G G G T C T G G G A C A
G Q A P R L L I Y A T S A L A S G I P D R F S G S G T
#175 1_DM.34.355_E05_09.ab1
G G C C A G G C T C C C A G G C T C T C A T C T A T G C C A C A T C C A C C T G G C T T C T G G C A T C C C A G A C A G G T T C A G T G G C A G T G G G T C T G G G A C A
G Q A P R L L I Y A T S N L A S G I P D R F S G S G T
#175 1_DM.35.355_D05_07.ab1
G G C C A G G C T C C C A G G C T C T C A T C T A T G C C C G T T C C G C T T G G C T T C T G G C A T C C C A G A C A G G T T C A G T G G C A G T G G G T C T G G G A C A
G Q A P R L L I Y A R S A L A S G I P D R F S G S G T
#175 1_DM.38.355_C05_05.ab1
G G C C A G G C T C C C A G G C T C T C A T C T A T G C C A C A T C C G C T C T G G C T T C T G G C A T C C C A G A C A G G T T C A G T G G C A G T G G G T C T G G G A C A
G Q A P R L L I Y A T S A L A S G I P D R F S G S G T
#175 1_DM.39.355_B05_03.ab1
G G C C A G G C T C C C A G G C T C T C A T C T A T G C C A C A T C C A C C T G G C T T C T G G C A T C C C A G A C A G G T T C A G T G G C A G T G G G T C T G G G A C A
G Q A P R L L I Y A T S N L A S G I P D R F S G S G T
#175 1_DM.40.355_A05_01.ab1
G G C C A G G C T C C C A G G C T C T C A T C T A T G C C A C A T C C A C C T G G C T T C T G G C A T C C C A G A C A G G T T C A G T G G C A G T G G G T C T G G G A C A
G Q A P R L L I Y A T S N L A S G I P D R F S G S G T

CONCISE

ContigVL
Sequencher(tm) '+'s ID10 comb1 10-10-02.SPF*

1010 VL	#349	GGCCAGGGGACCAAGCTGGAGATCAACGAACCTGTGGCTGCACCATCTGTCTTC																
	G	Q	G	T	K	L	E	I	K	R	T	V	A	A	P	S	V	F
1_DM.31.355_H05_15.ab1	#349	GGCCAGGGGACCAAGCTGGAGATCAACGAACCTGTGGCTGCACCATCTGTCTTC																
	G	Q	G	T	K	L	E	I	K	R	T	V	A	A	P	S	V	F
1_DM.32.355_G05_13.ab1	#349	GGCCAGGGGACCAAGCTGGAGATCAACGAACCTGTGGCTGCACCATCTGTCTTC																
	G	Q	G	T	K	L	E	I	K	R	T	V	A	A	P	S	V	F
1_DM.33.355_F05_11.ab1	#349	GGCCAGGGGACCAAGCTGGAGATCAACGAACCTGTGGCTGCACCATCTGTCTTC																
	G	Q	G	T	K	L	E	I	K	R	T	V	A	A	P	S	V	F
1_DM.34.355_E05_09.ab1	#349	GGCCAGGGGACCAAGCTGGAGATCAACGAACCTGTGGCTGCACCATCTGTCTTC																
	G	Q	G	T	K	L	E	I	K	R	T	V	A	A	P	S	V	F
1_DM.35.355_H05_07.ab1	#349	GGCCAGGGGACCAAGCTGGAGATCAACGAACCTGTGGCTGCACCATCTGTCTTC																
	G	Q	G	T	K	L	E	I	K	R	T	V	A	A	P	S	V	F
1_DM.36.355_C05_05.ab1	#349	GGCCAGGGGACCAAGCTGGAGATCAACGAACCTGTGGCTGCACCATCTGTCTTC																
	G	Q	G	T	K	L	E	I	K	R	T	V	A	A	P	S	V	F
1_DM.39.355_H05_03.ab1	#349	GGCCAGGGGACCAAGCTGGAGATCAACGAACCTGTGGCTGCACCATCTGTCTTC																
	G	Q	G	T	K	L	E	I	K	R	T	V	A	A	P	S	V	F
1_DM.40.355_A05_01.ab1	#349	GGCCAGGGGACCAAGCTGGAGATCAACGAACCTGTGGCTGCACCATCTGTCTTC																
	G	Q	G	T	K	L	E	I	K	R	T	V	A	A	P	S	V	F
<hr/>																		
	#349	GGCCAGGGGACCAAGCTGGAGATCAACGAACCTGTGGCTGCACCATCTGTCTTC																
	G	Q	G	T	K	L	E	I	K	R	T	V	A	A	P	S	V	F


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*+ 's 1D10 comb1 10-10-02.SPF"
sequencer (cm)
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1D10 VH	#1	GTGAACAACAAAGCACTATTGGCACTGGGCACTCTTACCGTTACTCTGTTTACACCTGTGCGAAAGCCGAGGTGCAGCTGCTGCAGCTCTGGAGCA
	V	K Q S T I A L A L L P L L F T F V A K A E V Q L V Q S G A
1_DM.31.530_H04_16.ab1	#1	GTGAACAACAAAGCACTATTGGCACTGGCACTCTTACCGTTACTGTTTACACCTGTGCGAAAGCCGAGGTGCAGCTGCTGCAGCTCTGGAGCA
	V	K Q S T I A L A L L F L L F T F V A K A E V Q L V Q S G A
1_DM.32.530_G04_14.ab1	#1	GTGAACAACAAAGCACTATTGGCACTGGCACTCTTACCGTTACTGTTTACACCTGTGCGAAAGCCGAGGTGCAGCTGCTGCAGCTCTGGAGCA
	V	K Q S T I A L A L L P L L F T F V A K A E V Q L V Q S G A
1_DM.33.530_F04_12.ab1	#1	GTGAACAACAAAGCACTATTGGCACTGGGCACTCTTACCGTTACTGTTTACACCTGTGCGAAAGCCGAGGTGCAGCTGCTGCAGCTCTGGAGCA
	V	K Q S T I A L A L L P L L F T F V A K A E V Q L V Q S G A
1_DM.34.530_E04_10.ab1	#1	GTGAACAACAAAGCACTATTGGCACTGGCACTCTTACCGTTACTGTTTACACCTGTGCGAAAGCCGAGGTGCAGCTGCTGCAGCTCTGGAGCA
	V	K Q S T I A L A L L P L L F T F V A K A E V Q L V Q S G A
1_DM.35.530_D04_08.ab1	#1	GTGAACAACAAAGCACTATTGGCACTGGCACTCTTACCGTTACTGTTTACACCTGTGCGAAAGCCGAGGTGCAGCTGCTGCAGCTCTGGAGCA
	V	K Q S T I A L A L L P L L F T F V A K A E V Q L V Q S G A
1_DM.38.530_C04_06.ab1	#1	GTGAACAACAAAGCACTATTGGCACTGGCACTCTTACCGTTACTGTTTACACCTGTGCGAAAGCCGAGGTGCAGCTGCTGCAGCTCTGGAGCA
	V	K Q S T I A L A L L P L L F T F V A K A E V Q L V Q S G A
1_DM.39.530_B04_04.ab1	#1	GTGAACAACAAAGCACTATTGGCACTGGCACTCTTACCGTTACTGTTTACACCTGTGCGAAAGCCGAGGTGCAGCTGCTGCAGCTCTGGAGCA
	V	K Q S T I A L A L L P L L F T F V A K A E V Q L V Q S G A
1_DM.40.530_A04_02.ab1	#1	GTGAACAACAAAGCACTATTGGCACTGGCACTCTTACCGTTACTGTTTACACCTGTGCGAAAGCCGAGGTGCAGCTGCTGCAGCTCTGGAGCA
	V	K Q S T I A L A L L P L L F T F V A K A E V Q L V Q S G A
<hr/>		
	#1	GTGAACAACAAAGCACTATTGGCACTGGCACTCTTACCGTTACTGTTTACACCTGTGCGAAAGCCGAGGTGCAGCTGCTGCAGCTCTGGAGCA
	V	K Q S T I A L A L L P L L F T F V A K A E V Q L V Q S G A

ContigVH
Sequencher (tm) "+"s 1D10 combi 10-10-02.SPF"

#91 1D10 VH #91 GAGGTGAAAAAGCCCCGGGAGTCTCTGAGATCTCTCTGTAAAGGTTCTGGCTACACATTACCAAGTTACATATATGCATGGGTGCGCCAG
E V K K P G E S L K I S C K G S G Y T F T S Y N M H W V R Q

#91 1.PM.31.530_H04_16.ab1 #91 GAGGTGAAAAAGCCCCGGGAGTCTCTGAGATCTCTCTGTAAAGGTTCTGGCTACACATTACCAAGTTACATATATGCATGGGTGCGCCAG
E V K K P G E S L K I S C K G S G Y T F T S Y N M H W V R Q

#91 1.PM.32.530_C04_14.ab1 #91 GAGGTGAAAAAGCCCCGGGAGTCTCTGAGATCTCTCTGTAAAGGTTCTGGCTACACATTACCAAGTTACATATATGCATGGGTGCGCCAG
E V K K P G E S L K I S C K G S G Y T F T S Y N M H W V R Q

#91 1.PM.33.530_F04_12.ab1 #91 GAGGTGAAAAAGCCCCGGGAGTCTCTGAGATCTCTCTGTAAAGGTTCTGGCTACACATTACCAAGTTACATATATGCATGGGTGCGCCAG
E V K K P G E S L K I S C K G S G Y T F T S Y N M H W V R Q

#91 1.PM.34.530_H04_10.ab1 #91 GAGGTGAAAAAGCCCCGGGAGTCTCTGAGATCTCTCTGTAAAGGTTCTGGCTACACATTACCAAGTTACATATATGCATGGGTGCGCCAG
E V K K P G E S L K I S C K G S G Y T F T S Y N M H W V R Q

#91 1.PM.35.530_J04_08.ab1 #91 GAGGTGAAAAAGCCCCGGGAGTCTCTGAGATCTCTCTGTAAAGGTTCTGGCTACACATTACCAAGTTACATATATGCATGGGTGCGCCAG
E V K K P G E S L K I S C K G S G Y T F T S Y N M H W V R Q

#91 1.PM.38.530_C04_06.ab1 #91 GAGGTGAAAAAGCCCCGGGAGTCTCTGAGATCTCTCTGTAAAGGTTCTGGCTACACATTACCAAGTTACATATATGCATGGGTGCGCCAG
E V K K P G E S L K I S C K G S G Y T F T S Y N M H W V R Q

#91 1.PM.39.530_E04_04.ab1 #91 GAGGTGAAAAAGCCCCGGGAGTCTCTGAGATCTCTCTGTAAAGGTTCTGGCTACACATTACCAAGTTACATATATGCATGGGTGCGCCAG
E V K K P G E S L K I S C K G S G Y T F T S Y N M H W V R Q

#91 1.PM.40.530_J04_02.ab1 #91 GAGGTGAAAAAGCCCCGGGAGTCTCTGAGATCTCTCTGTAAAGGTTCTGGCTACACATTACCAAGTTACATATATGCATGGGTGCGCCAG
E V K K P G E S L K I S C K G S G Y T F T S Y N M H W V R Q

#91 GAGGTGAAAAAGCCCCGGGAGTCTCTGAGATCTCTCTGTAAAGGTTCTGGCTACACATTACCAAGTTACATATATGCATGGGTGCGCCAG
E V K K P G E S L K I S C K G S G Y T F T S Y N M H W V R Q

ContigvH
Sequencher(tm) '+'s ID10 comb1 10-10-02.SPF"

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#181 1D10 VH ATGCCCCGGAAGGCGCTGAGATGGAATGGGGCTATTATCCCTTGATGCTGATCTTCTTACAAATCAGAAGTTCAAACTCCAGGTCACC
M P G K G L E W M G A I Y P L N G D T S Y N Q K F K L Q V T

#181 1.DM.31.530_H04_16.ab1 ATGCCCCGGAAGGCGCTGAGATGGAATGGGGCTATTATCCCTTGATGCTGATCTTCTTACAAATCAGAAGTTCAAACTCCAGGTCACC
M P G K G L E W M G A I Y P L N G D T S Y N Q K F K L Q V T

#181 1.DM.32.530_G04_14.ab1 ATGCCCCGGAAGGCGCTGAGATGGAATGGGGCTATTATCCCTTGATGCTGATCTTCTTACAAATCAGAAGTTCAAACTCCAGGTCACC
M P G K G L E W M G A I Y P L N G D T S Y N Q K F K L Q V T

#181 1.DM.33.530_F04_12.ab1 ATGCCCCGGAAGGCGCTGAGATGGAATGGGGCTATTATCCCTTGATGCTGATCTTCTTACAAATCAGAAGTTCAAACTCCAGGTCACC
M P G K G L E W M G A I Y P L N G D T S Y N Q K F K L Q V T

#181 1.DM.34.530_F04_10.ab1 ATGCCCCGGAAGGCGCTGAGATGGAATGGGGCTATTATCCCTTGATGCTGATCTTCTTACAAATCAGAAGTTCAAACTCCAGGTCACC
M P G K G L E W M G A I Y P L N G D T S Y N Q K F K L Q V T

#181 1.DM.35.530_H04_08.ab1 ATGCCCCGGAAGGCGCTGAGATGGAATGGGGCTATTATCCCTTGATGCTGATCTTCTTACAAATCAGAAGTTCAAACTCCAGGTCACC
M P G K G L E W M G A I Y P L N G D T S Y N Q K F K L Q V T

#181 1.DM.36.530_C04_06.ab1 ATGCCCCGGAAGGCGCTGAGATGGAATGGGGCTATTATCCCTTGATGCTGATCTTCTTACAAATCAGAAGTTCAAACTCCAGGTCACC
M P G K G L E W M G A I Y P L N G D T S Y N Q K F K L Q V T

#181 1.DM.39.530_E04_04.ab1 ATGCCCCGGAAGGCGCTGAGATGGAATGGGGCTATTATCCCTTGATGCTGATCTTCTTACAAATCAGAAGTTCAAACTCCAGGTCACC
M P G K G L E W M G A I Y P L N G D T S Y N Q K F K L Q V T

#181 1.DM.40.530_A04_02.ab1 ATGCCCCGGAAGGCGCTGAGATGGAATGGGGCTATTATCCCTTGATGCTGATCTTCTTACAAATCAGAAGTTCAAACTCCAGGTCACC
M P G K G L E W M G A I Y P L N G D T S Y N Q K F K L Q V T

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ContigVH
Sequencher(tm) '+'s ID10 combi 10-10-02.SPF"

#271 ID10 VH #271 ATCTCAGCCGACAGTCCATCTCAGCACCCGCTTACCTGCACTGGAGCAAGCCTTGAAAGCCTTCGAGACACCCGCCATGTATTACTGTGTGCGAGATCG
I S A D K S I S T A Y L Q W S S L K A S D T A M Y Y C A R S
#271 1_DM.31.530_H04_16.ab1 #271 ATCTCAGCCGACAGTCCATCTCAGCACCCGCTTACCTGCACTGGAGCAAGCCTTGAAAGCCTTCGAGACACCCGCCATGTATTACTGTGTGCGAGATCG
I S A D K S I S T A Y L Q W S S L K A S D T A M Y Y C A R S
#271 1_DM.32.530_G04_14.ab1 #271 ATCTCAGCCGACAGTCCATCTCAGCACCCGCTTACCTGCACTGGAGCAAGCCTTGAAAGCCTTCGAGACACCCGCCATGTATTACTGTGTGCGAGATCG
I S A D K S I S T A Y L Q W S S L K A S D T A M Y Y C A R S
#271 1_DM.33.530_F04_13.ab1 #271 ATCTCAGCCGACAGTCCATCTCAGCACCCGCTTACCTGCACTGGAGCAAGCCTTGAAAGCCTTCGAGACACCCGCCATGTATTACTGTGTGCGAGATCG
I S A D K S I S T A Y L Q W S S L K A S D T A M Y Y C A R S
#271 1_DM.34.530_F04_10.ab1 #271 ATCTCAGCCGACAGTCCATCTCAGCACCCGCTTACCTGCACTGGAGCAAGCCTTGAAAGCCTTCGAGACACCCGCCATGTATTACTGTGTGCGAGATCG
I S A D K S I S T A Y L Q W S S L K A S D T A M Y Y C A R S
#271 1_DM.35.530_D04_08.ab1 #271 ATCTCAGCCGACAGTCCATCTCAGCACCCGCTTACCTGCACTGGAGCAAGCCTTGAAAGCCTTCGAGACACCCGCCATGTATTACTGTGTGCGAGATCG
I S A D K S I S T A Y L Q W S S L K A S D T A M Y Y C A R S
#271 1_DM.38.530_C04_06.ab1 #271 ATCTCAGCCGACAGTCCATCTCAGCACCCGCTTACCTGCACTGGAGCAAGCCTTGAAAGCCTTCGAGACACCCGCCATGTATTACTGTGTGCGAGATCG
I S A D K S I S T A Y L Q W S S L K A S D T A M Y Y C A R S
#271 1_DM.39.530_F04_04.ab1 #271 ATCTCAGCCGACAGTCCATCTCAGCACCCGCTTACCTGCACTGGAGCAAGCCTTGAAAGCCTTCGAGACACCCGCCATGTATTACTGTGTGCGAGATCG
I S A D K S I S T A Y L Q W S S L K A S D T A M Y Y C A R S
#271 1_DM.40.530_A04_02.ab1 #271 ATCTCAGCCGACAGTCCATCTCAGCACCCGCTTACCTGCACTGGAGCAAGCCTTGAAAGCCTTCGAGACACCCGCCATGTATTACTGTGTGCGAGATCG
I S A D K S I S T A Y L Q W S S L K A S D T A M Y Y C A R S
#271 ATCTCAGCCGACAGTCCATCTCAGCACCCGCTTACCTGCACTGGAGCAAGCCTTGAAAGCCTTCGAGACACCCGCCATGTATTACTGTGTGCGAGATCG
I S A D K S I S T A Y L Q W S S L K A S D T A M Y Y C A R S

ContigvH
Sequencher(tm) '+'s ID10 comb1 10-10-02.SPf"

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#361 1D10 VH #361 ACTTACGTGGGCGGTGACTGGACTTTCGATGTCGTGGGGGTAAAGGAGCACGGGTGACGGTCTCTCCTCAAGCTTCGACCAAGGGCCC
T Y V G G D W T F D V W G K G T T V T V S S A S T K G
#361 1.DM.31.530_H04_16.ab1 #361 ACTTACGTGGGCGGTGACTGGAGTTGCGATGTCGTGGGGGTAAAGGAGCACGGGTGACGGTCTCTCCTCAAGCTTCGACCAAGGGCCC
T Y V G G D W Q F D V W G K G T T V T V S S A S T K G
#361 1.DM.32.530_G04_14.ab1 #361 ACTTACGTGGGCGGTGACTGGAGTTGCGATGTCGTGGGGGTAAAGGAGCACGGGTGACGGTCTCTCCTCAAGCTTCGACCAAGGGCCC
T Y V G G D W Q F D V W G K G T T V T V S S A S T K G
#361 1.DM.33.530_F04_12.ab1 #361 ACTTACGTGGGCGGTGACTGGAGTTGCGATGTCGTGGGGGTAAAGGAGCACGGGTGACGGTCTCTCCTCAAGCTTCGACCAAGGGCCC
T Y V G G D W Q F D V W G K G T T V T V S S A S T K G
#361 1.DM.34.530_H04_10.ab1 #361 ACTTACGTGGGCGGTGACTGGAGTTGCGATGTCGTGGGGGTAAAGGAGCACGGGTGACGGTCTCTCCTCAAGCTTCGACCAAGGGCCC
T Y V G G D W T F D V W G K G T T V T V S S A S T K G
#361 1.DM.35.530_D04_08.ab1 #361 ACTTACGTGGGCGGTGACTGGAGTTGCGATGTCGTGGGGGTAAAGGAGCACGGGTGACGGTCTCTCCTCAAGCTTCGACCAAGGGCCC
T Y V G G D W Q F D V W G K G T T V T V S S A S T K G
#361 1.DM.38.530_C04_06.ab1 #361 TATTACGTGGGCGGTGACTGGAGTTGCGATGTCGTGGGGGTAAAGGAGCACGGGTGACGGTCTCTCCTCAAGCTTCGACCAAGGGCCC
Y Y V G G D W Q F D V W G K G T T V T V S S A S T K G
#361 1.DM.39.530_H04_04.ab1 #361 ACTTACGTGGGCGGTGACTGGAGTTGCGATGTCGTGGGGGTAAAGGAGCACGGGTGACGGTCTCTCCTCAAGCTTCGACCAAGGGCCC
T Y V G G D W T F D V W G K G T T V T V S S A S T K G
#361 1.DM.40.530_A04_02.ab1 #361 ACTTACGTGGGCGGTGACTGGAGTTGCGATGTCGTGGGGGTAAAGGAGCACGGGTGACGGTCTCTCCTCAAGCTTCGACCAAGGGCCC
T Y V G G D W Q F D V W G K G T T V T V S S A S T K G
#361 ACTTACGTGGGCGGTGACTGGAGTTGCGATGTCGTGGGGGTAAAGGAGCACGGGTGACGGTCTCTCCTCAAGCTTCGACCAAGGGCCC
T Y V G G D W Q F D V W G K G T T V T V S S A S T K G

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COCTIQUIN

EXHIBIT 4

ContigVL
Sequencher(tm) '+'s 1D10 comb1 10-10-02.SPF"

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#88 1D10 VL CTGTCCTTTGTCCTCCAGGGGAAAGAGCCACCCCTCTCTCTCTGCAAGGGCCCACTTCAGTGTACCGGTACATCCACTGGGTACCAAGCAAAACCT
L S L S P G E R A T L S C R A S S S V P Y I H W Y Q Q K P

#88 1_DM.31.355_H05_15.ab1 CTGTCCTTTGTCCTCCAGGGGAAAGAGCCACCCCTCTCTCTCTGCAAGGGCCCACTTCAGTGTACCGGTACATCCACTGGGTACCAAGCAAAACCT
L S L S P G E R A T L S C R A S S S V P Y I H W Y Q Q K P

#88 1_DM.32.355_G05_13.ab1 CTGTCCTTTGTCCTCCAGGGGAAAGAGCCACCCCTCTCTCTCTGCAAGGGCCCACTTCAGTGTACCGGTACATCCACTGGGTACCAAGCAAAACCT
L S L S P G E R A T L S C R A S S S V P Y I H W Y Q Q K P

#88 1_DM.33.355_F05_11.ab1 CTGTCCTTTGTCCTCCAGGGGAAAGAGCCACCCCTCTCTCTCTGCAAGGGCCCACTTCAGTGTACCGGTACATCCACTGGGTACCAAGCAAAACCT
L S L S P G E R A T L S C R A S S S V P Y I H W Y Q Q K P

#88 1_DM.34.355_H05_09.ab1 CTGTCCTTTGTCCTCCAGGGGAAAGAGCCACCCCTCTCTCTCTGCAAGGGCCCACTTCAGTGTACCGGTACATCCACTGGGTACCAAGCAAAACCT
L S L S P G E R A T L S C R A S S S V P Y I H W Y Q Q K P

#88 1_DM.35.355_D05_07.ab1 CTGTCCTTTGTCCTCCAGGGGAAAGAGCCACCCCTCTCTCTCTGCAAGGGCCCACTTCAGTGTACCGGTACATCCACTGGGTACCAAGCAAAACCT
L S L S P G E R A T L S C R A S S S V P Y I H W Y Q Q K P

#88 1_DM.38.355_C05_05.ab1 CTGTCCTTTGTCCTCCAGGGGAAAGAGCCACCCCTCTCTCTCTGCAAGGGCCCACTTCAGTGTACCGGTACATCCACTGGGTACCAAGCAAAACCT
L S L S P G E R A T L S C R A S S S V P Y I H W Y Q Q K P

#88 1_DM.39.355_H05_03.ab1 CTGTCCTTTGTCCTCCAGGGGAAAGAGCCACCCCTCTCTCTCTGCAAGGGCCCACTTCAGTGTACCGGTACATCCACTGGGTACCAAGCAAAACCT
L S L S P G E R A T L S C R A S S S V P Y I H W Y Q Q K P

#88 1_DM.40.355_A05_01.ab1 CTGTCCTTTGTCCTCCAGGGGAAAGAGCCACCCCTCTCTCTCTGCAAGGGCCCACTTCAGTGTACCGGTACATCCACTGGGTACCAAGCAAAACCT
L S L S P G E R A T L S C R A S S S V P Y I H W Y Q Q K P

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ContigVL
Sequencher (cm) "+"s 1D10 combi 10-10-02.SPF"

#175 1D10 VL
G G C C A G G C T C C C A G G C T T C A T C T A T G C C A C A T C C A C C T G G C T T C T G G C A T C C C A G A C A G G T T C A G T G G C A G T G G G T C T G G G A C A
G Q A P R L L I Y A T S N L A S G I P D R F S G S G T
#175 1_DM.31.355_H05_15.ab1
G G C C A G G C T C C C A G G C T C C A T C T A T G C C C G T T C C A A C C T G G C T T C T G G C A T C C C A G A C A G G T T C A G T G G C A G T G G G T C T G G G A C A
G Q A P R L L I Y A R S N L A S G I P D R F S G S G T
#175 1_DM.32.355_G05_13.ab1
G G C C A G G C T C C C A G G C T C C A T C T A T G C C A C A T C C G C T C T G G C T T C T G G C A T C C C A G A C A G G T T C A G T G G C A G T G G G T C T G G G A C A
G Q A P R L L I Y A T S A L A S G I P D R F S G S G T
#175 1_DM.33.355_F05_11.ab1
G G C C A G G C T C C C A G G C T C C A T C T A T G C C A C A T C C G C C T T G G C T T C T G G C A T C C C A G A C A G G T T C A G T G G C A G T G G G T C T G G G A C A
G Q A P R L L I Y A T S A L A S G I P D R F S G S G T
#175 1_DM.34.355_E05_09.ab1
G G C C A G G C T C C C A G G C T C C A T C T A T G C C A C A T C C A C C T G G C T T C T G G C A T C C C A G A C A G G T T C A G T G G C A G T G G G T C T G G G A C A
G Q A P R L L I Y A T S N L A S G I P D R F S G S G T
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G Q A P R L L I Y A T S A L A S G I P D R F S G S G T
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G Q A P R L L I Y A T S N L A S G I P D R F S G S G T
#175 1_DM.40.355_A05_01.ab1
G G C C A G G C T C C C A G G C T C C A T C T A T G C C A C A T C C A C C T G G C T T C T G G C A T C C C A G A C A G G T T C A G T G G C A G T G G G T C T G G G A C A
G Q A P R L L I Y A T S N L A S G I P D R F S G S G T

CONCISE

#262 GACTTCACCTCTCCACCATCAGCAGACTGGAGCCCTGAGAGATTTTTCCACAGTGATTTACTGTCAACCACTGSGCTGAGTACCCACCCCACTTTT
D F T L T T I S R L E P E D F A V Y Y C Q Q W L S N P P T F

ContigVL
Sequencher(tm) '+'s ID10 comb1 10-10-02.SPF*

1010 VL	#349	GGCCAGGGGACCAAGCTGGAGATCAACGAACCTGTGGCTGCACCATCTGTCTTC																
	G	Q	G	T	K	L	E	I	K	R	T	V	A	A	P	S	V	F
1_DM.31.355_H05_15.ab1	#349	GGCCAGGGGACCAAGCTGGAGATCAACGAACCTGTGGCTGCACCATCTGTCTTC																
	G	Q	G	T	K	L	E	I	K	R	T	V	A	A	P	S	V	F
1_DM.32.355_G05_13.ab1	#349	GGCCAGGGGACCAAGCTGGAGATCAACGAACCTGTGGCTGCACCATCTGTCTTC																
	G	Q	G	T	K	L	E	I	K	R	T	V	A	A	P	S	V	F
1_DM.33.355_F05_11.ab1	#349	GGCCAGGGGACCAAGCTGGAGATCAACGAACCTGTGGCTGCACCATCTGTCTTC																
	G	Q	G	T	K	L	E	I	K	R	T	V	A	A	P	S	V	F
1_DM.34.355_E05_09.ab1	#349	GGCCAGGGGACCAAGCTGGAGATCAACGAACCTGTGGCTGCACCATCTGTCTTC																
	G	Q	G	T	K	L	E	I	K	R	T	V	A	A	P	S	V	F
1_DM.35.355_H05_07.ab1	#349	GGCCAGGGGACCAAGCTGGAGATCAACGAACCTGTGGCTGCACCATCTGTCTTC																
	G	Q	G	T	K	L	E	I	K	R	T	V	A	A	P	S	V	F
1_DM.36.355_C05_05.ab1	#349	GGCCAGGGGACCAAGCTGGAGATCAACGAACCTGTGGCTGCACCATCTGTCTTC																
	G	Q	G	T	K	L	E	I	K	R	T	V	A	A	P	S	V	F
1_DM.39.355_H05_03.ab1	#349	GGCCAGGGGACCAAGCTGGAGATCAACGAACCTGTGGCTGCACCATCTGTCTTC																
	G	Q	G	T	K	L	E	I	K	R	T	V	A	A	P	S	V	F
1_DM.40.355_A05_01.ab1	#349	GGCCAGGGGACCAAGCTGGAGATCAACGAACCTGTGGCTGCACCATCTGTCTTC																
	G	Q	G	T	K	L	E	I	K	R	T	V	A	A	P	S	V	F
<hr/>																		
	#349	GGCCAGGGGACCAAGCTGGAGATCAACGAACCTGTGGCTGCACCATCTGTCTTC																
	G	Q	G	T	K	L	E	I	K	R	T	V	A	A	P	S	V	F

EXHIBIT 5

P.28-97 CD20 8-22-02 to 11-7-02

NOTEBOOK NO. 585
ISSUED TO Chris Hawelka
ON 6-11 2002
DEPARTMENT _____
RETURNED _____ 20 _____

SCIENTIFIC NOTEBOOK COMPANY
2831 LAWRENCE AVENUE
STEVENSVILLE, MICHIGAN 49127
(800) 537-3028 - <http://www.snco.com>

Project No. CD-20Book No. 3

TITLE

AntigenicHT+SSSet up15ml per3 Rema plates

From Page No. _____

10/8 Antigenic prep on 31-40

elute of 150-TEs

dilute per prep 1:3 71% BSA-PBS
+ from assay C1 #31-40

10/8 Bone set up HT+SS on 31-40

31 E2

36 FH

32 E2

37 DT

33 F1

38 B1

34 G1

39 FH

35 B1

40 C9

Finished on 10/9

10/9 Grow X10's for 15ml per prep

300 ml 2X YT 9-26

300 ml Tet

3 ml X10 10/9

mix & grow @ 37°C shaker

Check OD 595 $\approx 2.45 \times 10^8$ 0.403Add 300 ml 1M IPTG + ~~mix~~ mix

pilot 15ml tubes

Add 5% HT from #31-40 above

+ New Chi

41-1010

Growth 4#5

Quanta

Chi 12.90

21011m 13.00

41-1010 27.12

Growth 4#5 7.58

14 55.94

15 38.35

17 37.96

18 42.59

19 48.30

21 66.44

22 52.25

23 43.68

24 37.06

25 41.71

26 69.83

27 48.51

28 47.52

29 59.73

Chi 12.95

41-1010 38.84

10/9 Set 3 Rema plates

Dead $3 \times 10^5 = 13/2 \times 2 (1 \times 10^4) = 1.3 \times 10^5$ deadLive $506 + 609 = 1135 \times 13 = (122/2 \times 10^4) 1 \times 10^4 = 1.1 \times 10^7$

$$\frac{1.3 \times 10^5}{1.1 \times 10^7} = \frac{1.21}{1.1} \left\{ \left(\frac{0.011819}{1} \right) \times 100 \right\} - 100 \quad (99.8\% \text{ viability})$$

Set 3 Quant plates

10ml Carbonate buffer + 3.92 α fd mix & pipet 50 μ l well
to poly D type plate, store @ 4°C ON

To Page No. _____

Witnessed & Understood by me.

Date

Initialed by

Christine M. Hamblin

Date

10-9-02

Recorded by

From Page No.

10/10 Finished 15 ml series for #31-40

Did 2 Ramon Assays + 3 Quanta

Quanta used 100 fold dilution program - to get absolute Fab core #2

New Chi - 25.00 ng/ml / 25.00

L1-1010 59.02 29.51

Shin's 4H5 19.85 7.425

30-H1 89.05 44.535

31-E2 59.44 29.72

32-E2 44.51 22.255

33-A1 74.48 37.24

34-G1 71.75 35.875

35-B1 70.86 35.43

36-F4 110.20 55.10

37-D8 75.12 ng/ml

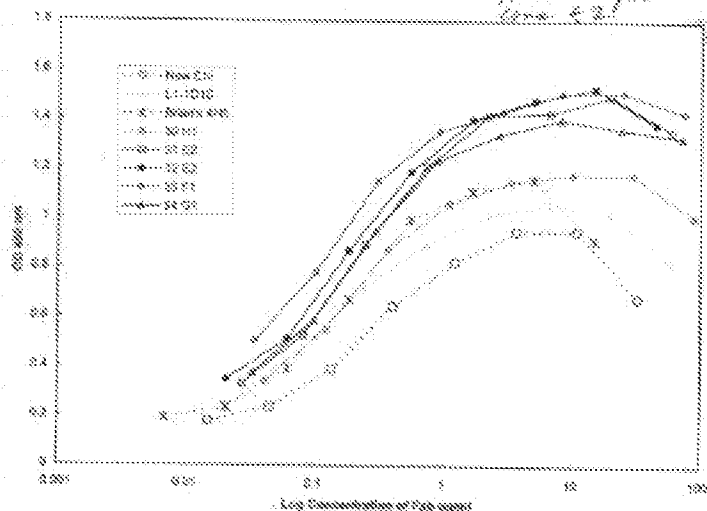
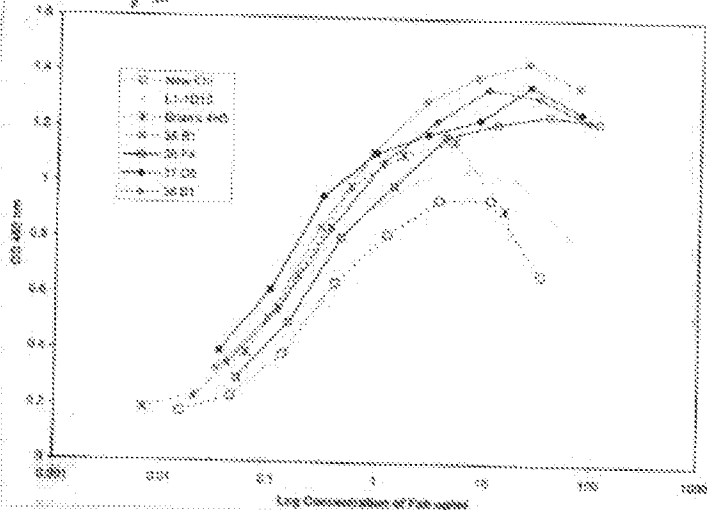
38-B1 88.77 44.385

39-F4 68.42 34.21

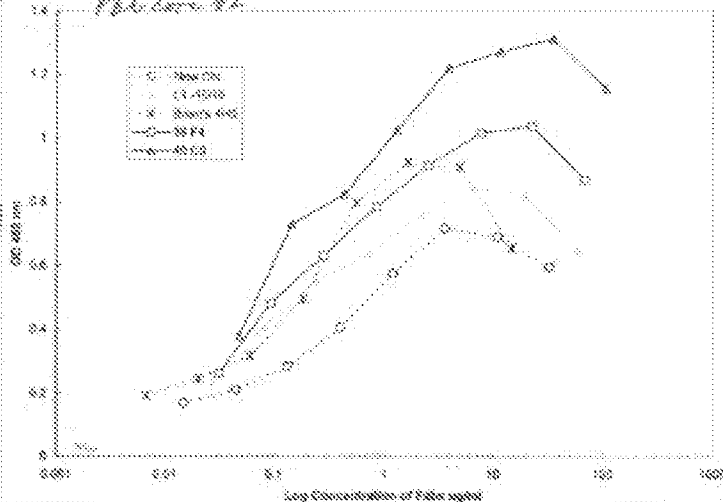
New Chi 40.08 20.04

40-C9 105.28 52.64

Binding of Fab to Fixed Ramon Cells 10-10-02

absolute Fab
Core #2absolute Fab Binding of Fab to Fixed Ramon Cells 10-10-02
Core #2

absolute Fab core #2 Binding of Fab to Fixed Ramon Cells 10-10-02



1010 is slightly above Chi in all these graphs (didn't show binding in yellow) - corrected - printed out new graphs.

#32, 33, 35 + 40 looks the best, so will investigate them further.

To Page No.

Witnessed & Understood by me,

Date

Initiated by

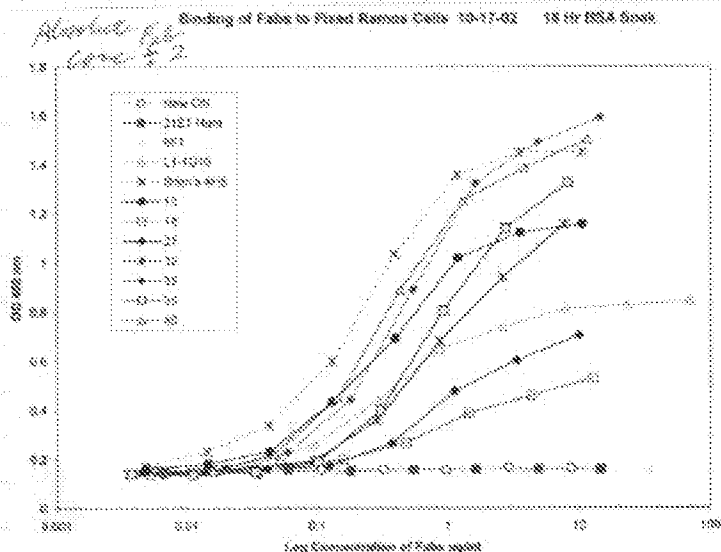
Christina K. Havelka

Date

10-10-02

Recorded by

From Page No. _____



- 18 hr. - Gave Kinase 9461 as well as ~~27~~ 40 staying on pretty well

- Cytocin, 6F1 + 21E1 Hum are off

- 18 has come off the most
~~27 + 40~~
27 + 1010 have also fallen off a little

10/17 Kinase 9461 D101N 65 nM 841 ng/ml

template ~ 9000 bases $\times 330 = 2,970,000$ M

oligo 39 bases $\times 330 = 12,870$ M

$2,970,000 / 12,870 = 230.77$ or 231x's

$9000 / 39 = 230$'s too much if 160 ng each

9000 template	160 ng	160 ng	160 ng	160 ng	160 ng	160 ng
39 oligo	160 ng	160 ng	8 ng	8 ng	7 ng	7 ng
too much	230x's too much	23x's	11.5x's	10x's	9x's	9x's

about right

So want 5 ng of oligo in 12 μ l $\Rightarrow 20 \times 5 \text{ ng} = 100 \text{ ng}$ $\Rightarrow 100 \text{ ng} / 12 \mu\text{l} = 8.33 \text{ ng}/\mu\text{l}$

Conc. 841 ng/ml $\Rightarrow 841 \text{ ng}/\mu\text{l}$
84.1 ng/ μ l
1:10 dilution of sterile H₂O
740 ng / 84.1 = 1.662 μ l of oligo

2 x 10x Phosphatidylcholine Buffer
1x 10 mM ATP 4-18-00
1.662 oligo 9461
14.37 x sterile H₂O
.5 x Kinase
20 x total
1 hr @ 37°C

To Page No. _____

Witnessed & Understood by me.

Date

Initialed by

Christine M. Hume

Date

10-17-02

Recorded by

NOTEBOOK NO. 613
ISSUED TO Ying Nie
ON 9/17/02 20
DEPARTMENT _____
RETURNED _____ 20

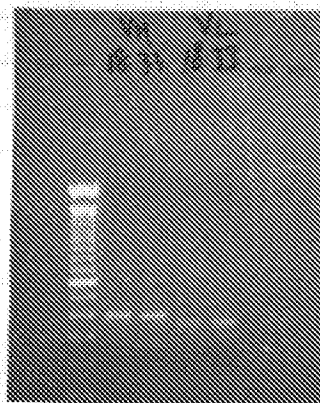
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From Page No. _____

1. get PCR product from YT
~100 μ l/sample

VH \leq 18
33

VL \leq 18
33



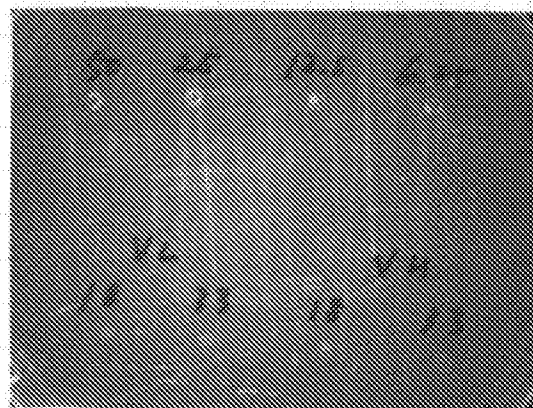
2. PCR product purification

- + 500 μ l buf 1 (binding buf) to PCR product \rightarrow column
- + 500 μ l buf 2 (washing buf) \times 2
- + 200 μ l " "
- + 50 μ l buf 3 (elute buf) \times 2
- then + 150 μ l dH₂O

3. to each sample.

- 150 μ l of Magnetic beads + 400 μ l 2xRLW buf — wash \times 2
- resuspend in 200 μ l 2xRLW buf
- beads + DNA gently shaking 16'
- wash beads w/ 2xRLW buf \times 4
- elute DNA in 300 μ l of 0.15M NaOH, gently shaking 10'
- take out, \times 2 to remove residual beads
- + 1 μ l glycogen
- 30 μ l 3M NaOAc (pH 5.0) / -80°C — 30'
- 800 μ l 100% EtOH
- \times 2, 70% EtOH wash, vac dry, dissolve in 15 μ l dH₂O

Keep at -20°C (YT refre.)



To Page No. _____

Witnessed & Understood by me.

Date

Invented by

Date

Carolyn Ho

6.1.03

Recorded by

Ying Nie

11/4/02